510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k120945

B. Purpose for Submission:

New devices

C. Measurand:

ALT (Alanine aminotransferase)

AST (Aspartate aminotransferase)

D. Type of Test:

Quantitative Photometric

E. Applicant:

Hitachi Chemical Diagnostics, Inc.

F. Proprietary and Established Names:

S TEST Reagent Cartridge Alanine aminotransferase (ALT)

S TEST Reagent Cartridge Aspartate aminotransferase (AST)

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.1030 - ALT test system
   21 CRF §862.1100 - AST test system

2. Classification:

   Class I, exempt, meets the limitation of exemptions in 862.9(c)(9)

   Class II

3. Product code:

   CKA; CIT

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indication(s) for use below

2. Indication(s) for use:

   The Hitachi Clinical Analyzer with S TEST Reagent Cartridge for ALT is intended for the quantitative measurement of the activity of the enzyme alanine amino transferase (ALT) in serum, lithium heparin plasma, K3 EDTA plasma, and sodium citrate plasma. The test system is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only. ALT measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.

   The Hitachi Clinical Analyzer with S TEST Reagent Cartridge for AST is intended for the quantitative measurement of the activity of the enzyme aspartate amino transferase (AST) in serum, lithium heparin plasma, K3 EDTA plasma, and sodium citrate plasma. The test system is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only. AST measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.

3. Special conditions for use statement(s):

   Prescription use only

   For clinical laboratories and Point of Care settings.

4. Special instrument requirements:

   Hitachi Clinical Analyzer E40

I. Device Description:

The S TEST reagent cartridges are made of plastic and include two small reservoirs capable of holding two separate reagents (R1 and R2), separated by a reaction cell/photometric cuvette. The cartridges also include a dot code label that contains all chemistry parameters, calibration factors, and other production-related information, e.g., expiration dating. The dimensions of the reagent cartridges are: 13.5 mm (W) × 28 mm (D) × 20.2 mm (H).

ALT Reagent #1:

- Lactate dehydrogenase (isolated from chicken heart) 3.1 U/mL
- Nicotinamide adenine dinucleotide (reduced form) 0.34 mmol/L
- L-alanine 180 mmol/L

ALT Reagent #2:

- L-alanine 730 mmol/L
- Alfa-ketoglutaric acid 47 mmol/L

AST Reagent #1:

- Nicotinamide adenine dinucleotide (reduced form) 0.25 mmol/L

- Malate dehydrogenase (derived from Thermus) 0.78 U/mL
- 2-Amino-2-hydroxymethyl-1,3-propanediol buffer (pH 7.8) 80 mmol/L
- L-Aspartic acid 104 mmol/L

AST Reagent #2:

- L-Aspartic acid 415 mmol/L
- Alfa-Ketoglutaric acid 31 mmol/L
- 2-Amino-2-hydroxymethyl-1,3-propanediol buffer (pH 7.8) 80 mmol/L

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Sekisui ALT-SL Assay for ALT

   Roche/Hitachi cobas 8000 (c502 module) - for AST.

2. Predicate 510(k) number(s):

   k974003

   k100853

3. Comparison with predicate:

   A comparative chart detailing the similarities and differences among the various test systems is shown below:

ALT Test System	**Hitachi E40 ALT S Test System - k120945 (Candidate Device)**	**Sekisui ALT-SL Assay k974003 (Predicate Device)**
Intended Use/Indications for use	For the quantitative determination of ALT in human serum. ALT measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.	Same
Testing Environment	Physician office or clinical lab	Clinical lab
Test Principle	NADH oxidation of pyruvate formed by L-alanine and alpha-ketoglutarate in the presence of ALT	NADH oxidation of pyruvate formed by L-alanine and 2-oxyglutarate in the presence of ALT
Detection Wavelength	340/546 nm	340/415 nm
Specimen Type	Human serum or plasma	Human serum or plasma
Reportable Range	6.0 to 400 U/L	10.0 to 600 U/L
Precision	%CVs range from 2.3% to 5.6%	%CVs range from 2.4% to 3.6%

AST Test System	**Hitachi E40 AST S Test System - k120945 (Candidate Device)**	**Roche cobas 8000 AST - K100853 (Predicate Device)**
Intended Use/Indications for Use	For the quantitative determination of AST in human serum. AST measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.	Same
Testing Environment	Physician office or clinical lab	Clinical lab
Test Principle	NADH oxidation of pyruvate formed by L-aspartate and alpha-ketoglutarate in the presence of AST	NADH oxidation of pyruvate formed by L-aspartate and 2-oxyglutarate in the presence of AST
Detection Wavelength	340/546 nm	700/340 nm
Specimen Type	Human serum or plasma	Human serum or plasma
Reportable Range	4.0 to 400 U/L	5.0 to 700 U/L
Precision	%CVs range from 1.4% to 3.2%	%CVs range from 0.4% to 3.1%

K. Standard/Guidance Document referenced (if applicable):

CLSI EP5-A2, "Evaluation of Precision Performance of Clinical Chemistry Devices"

CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement"

CLSI EP7-A2, "Interference Testing in Clinical Chemistry"

CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation"

L. Test Principle:

Alanine aminotransferase (ALT) catalyzes the reaction from L-alanine and alfa-ketoglutaric acid to pyruvic acid and glutamic acid. When the produced pyruvic acid is converted into lactic acid by lactate dehydrogenase (LD), NADH is converted into NAD with a decrease in absorbance at 340 nm. The ALT activity can be determined by measuring the decreased rate of NADH.

$$\text{L-Alanine + Alfa-Ketoglutaric acid} \xrightarrow{\text{ALT}} \text{Pyruvic acid + Glutamic acid}$$

$$\text{Pyruvic acid + NADH} \xrightarrow{\text{LD}} \text{Lactic acid + NAD}$$

Aspartate aminotransferase (AST) catalyzes a reaction from L-aspartic acid and α-ketogulutaric acid to oxaloacetic acid and glutamic acid. When the produced oxaloacetic acid is converted into malic acid by malate dehydrogenase (MD), NADH is converted into NAD with a decrease in absorbance at 340 nm. The AST activity can be determined by measuring the decreased rate of NADH.

$$\text{L-Aspartic acid + Alfa-Ketoglutaric acid} \xrightarrow{\text{AST}} \text{Oxaloacetic acid + Glutamic acid}$$

$$\text{Oxaloacetic acid + NADH} \xrightarrow{\text{MD}} \text{Malic acid + NAD}$$

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

20-day In-house Precision

The studies followed CLSI EP5-A2, where three levels of samples were each tested in duplicate, twice a day, for 20 days, for a total of 80 results per level. The low and intermediate level samples (level 1 and level 2) were commercial controls; the high level (level 3) samples were archived (stored frozen at -20ºC) patient serum samples. The results are as follows:

ALT		Mean (U/L)	Within-Run %CV	Total %CV
N = 80 per level	Level 1	25.3	4.3	5.6
	Level 2	79.6	1.5	2.7
	Level 3	286.7	1.2	2.3

AST		Mean (U/L)	Within-Run %CV	Total %CV
N = 80 per level	Level 1	41.5	2.0	3.2
	Level 2	109.6	1.1	1.4
	Level 3	304.2	0.9	3.0

Physician Office Precision Study at Sites 1, 2 and 3

Studies for precision were performed at three external POL-type sites to evaluate the Hitachi Clinical Analyzer with S TEST ALT and AST reagent cartridges in one of its targeted intended use environments, the physician's office laboratory.

For the external site precision study, each site received three blinded serum samples that were chosen to represent low, intermediate, and high concentrations of each analyte. The samples at Sites 1 and 2 (Samples A, B, and C) were spiked serum specimens where the neat sera were collected under IRB approvals and were processed by routine procedures, frozen to -20ºC, and shipped frozen to each site. The samples at Site 3 (Samples D, E, and F) were commercial controls targeted to lower levels. Each sample was assayed six times per day for five days, reporting 30 results per level per analyte. Precision estimates for within-run precision and total precision were as follows:

ALT, N = 30 replicates per sample per site

POL Site	Sample	Mean (U/L)	Within-run Precision		Total Precision	
			SD (U/L)	%CV	SD (U/L)	%CV
Site 1	A	51.8	2.0	3.9%	2.0	3.9%
Site 2	A	51.7	2.4	4.7%	2.4	4.7%
Site 3	D	24.3	0.9	3.8%	1.4	5.7%
Site 1	B	143.8	2.2	1.5%	3.4	2.4%
Site 2	B	139.5	2.5	1.8%	2.8	2.0%
Site 3	E	77.4	1.1	1.4%	2.0	2.6%
Site 1	C	319.7	3.8	1.2%	5.0	1.6%

Site 2	C	305.9	4.1	1.3%	7.7	2.5%
Site 3	F	194.4	2.2	1.1%	4.3	2.2%

AST, N = 30 replicates per sample per site

Site	Sample	Mean (U/L)	Within-run Precision		Total Precision	
			SD (U/L)	%CV	SD (U/L)	%CV
Site 1	A	76.3	1.9	2.5%	2.0	2.7%
Site 2	A	75.4	1.7	2.2%	1.6	2.1%
Site 3	D	41.0	0.9	2.2%	0.9	2.1%
Site 1	B	156.8	2.1	1.3%	3.8	2.4%
Site 2	B	154.4	2.5	1.6%	4.1	2.7%
Site 3	E	106.1	0.9	0.8%	2.0	1.9%
Site 1	C	356.0	5.8	1.6%	6.9	1.9%
Site 2	C	348.9	7.3	2.1%	20.6	5.9%
Site 3	F	256.9	2.6	1.0%	4.9	1.9%

b. Linearity/assay reportable range:

The studies followed CLSI EP-6A. Nine to 10 serial dilutions per analyte assay system were prepared using the commercially available linearity/calibration set. The calibration samples were assigned their reference values arithmetically from the labeled values and were tested in duplicate by the Hitachi Clinical Analyzer. The mean Hitachi results (y-axis) were plotted against the assigned values (x-axis). The ALT S TEST is linear between 6 and 400 U/L and the AST S Test is linear between 4 and 400 U/L.

Analyte	Linearity Range Tested (U/L)	Regression Analysis	Reportable Range Claimed (U/L)
ALT	0.7 to 438.0	$y = 0.9899x - 1.6095$ $R^2 = 0.9998$	6.0 to 400
AST	2.4 to 525.7	$y = 1.0244x - 3.7708$ $R^2 = 0.9998$	4.0 to 400

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:

Each lot of S TEST cartridges (for both ALT and AST) is calibrated by the manufacturer prior to shipment using material traceable to Japanese Enzyme Reference Material (JCCLS CRM-001). The 2D code printed on each cartridge provides the analyzer with lot-specific calibration data.

Cartridge Shelf-Life/Stability Study:

Real-time, shelf-life stability studies for the ALT and AST reagent cartridges were performed. A two-level control set [37.8 U/L (normal) and 133.3 U/L (abnormal)] was tested in replicates of five with three lots of cartridges for both ALT and AST

across six analyzers according to standard procedure. Testing was performed at Time 0 (baseline), and at approximately, 6, 11, 12 and 13 months; the storage condition was refrigerated (2 to 8 ºC). The acceptance criterion at each time point was 90% to 110% recovery for each sample, using the label value of the controls as the target value.

Based on the results, the shelf life for both the ALT and the AST reagent systems is 12 months, and stability at the 12 month time point met the specification in every case.

d. Detection limit:

The studies followed CLSI EP17. For the LoB, the blank sample for each reagent system was assayed 20 times per day for three days for a total of 60 replicate results. The LoB was estimated as the mean of the 57th and 58th highest values for the true blanks. For the LoD, five low samples were assayed four times per day for three days, for a total of 60 replicate results. The LoD was calculated as the LoB + 1.645 x (SD of the low sample). The ALT limit of detection (LoD) is 2.2 U/L, and the LOD for AST is 1.4 U/L. For the estimation of LoQ, six samples ranging in concentration between 5.0 and 9.9 U/L for ALT and between 3.8 to 8.8 U/L for AST were tested six times a day for three days on three analyzers with one lot of respective reagent cartridges (n=54). For ALT, the calculated means were 7.6, 7.9, 9.9, 5.0, 6.1 and 5.3 U/L with %CV ranging from 7.4 to 19.0%. Based on the results, the LoQ for the ALT assay was estimated as 6.0 U/L. Similarly for AST, the calculated means were 8.8, 6.9, 8.6, 3.8, 5.9 and 5.6 U/L with %CV ranging from 5.9 to 20.4%. Based on the results, the LoQ was estimated as 4.0 U/L.

Limits	**ALT**	**AST**
LoB	0.8 U/L	0.5 U/L
LoD	2.2 U/L	1.4 U/L
LoQ	6.0 U/L	4.0 U/L

The results of the LoQ studies support the sponsor's claimed measuring range for ALT between 6.0 to 400 U/L and for AST between 4.0 to 400 U/L.

e. Analytical specificity:

The studies followed CLSI EP7-A2. The study evaluated increasing concentrations of potential endogenous interferents (ascorbic acid, unconjugated bilirubin, triglyceride and hemoglobin) in the ALT and AST assays. Two levels of commercial control sera at approximately 40 and 100 U/L ALT and AST concentrations were spiked with six levels of interferents and tested in replicates of three. In each case, the spiked sample result mean was compared to its neat control mean result, and percent recoveries were calculated. The data demonstrated that the S TEST for ALT and the S Test for AST were not affected by high levels of the following substances at the levels noted below. The interference claim (no significant interference) for each

analyte is defined by the sponsor as the highest level of interferent that is within 10% of the neat sample.

ALT

- Hemoglobin: no interference up to 250 mg/dL
- Unconjugated bilirubin: no interference up to 25 mg/dL
- Triglyceride: no interference up to 500 mg/dL
- Ascorbic acid: no interference up to 50 mg/dL

The sponsor has included the following statement in the ALT assay labeling:
"Use clear, unhemolyzed serum or plasma"

AST

- Hemoglobin: no interference up to 31 mg/dL
- Unconjugated bilirubin: no interference up to 50 mg/dL
- Triglyceride: no interference up to 500 mg/dL
- Ascorbic acid: no interference up to 50 mg/dL

Since hemoglobin showed significant interference for AST, the sponsor has included the following statement in the AST assay labeling:
"Use clear, unhemolyzed serum or plasma; avoid even slight hemolysis"

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies evaluated approximately 100 serum samples.

For ALT, matched aliquots from 103 clinical specimens spanning the measuring range were assayed in singleton by both the S Test ALT method and the Sekisui method. The data were analyzed by linear regression (S Test ALT = y-axis), and the results were as follows:

ALT (U/L)
N = 103 ; Less than 10% of the samples were altered samples (diluted)
sample range = 12 to 392 U/L
y= 1.0868x + 2.339
correlation coefficient (R^2) = 0.9987
95% confidence interval of the slope = 1.08 to 1.09;
95% confidence interval of the y-intercept = 1.4 to 3.3

For AST, matched aliquots from 169 samples spanning the measuring range were assayed in singleton by both the Hitachi Clinical Analyzer with S TEST AST assay

and the Roche/Hitachi cobas 6000 AST assay. The data were analyzed by linear regression (S Test AST = y-axis), and the results were as follows:

AST (U/L)
N = 169; Less than 10% of the samples are altered samples (diluted or spiked)
sample range = 5 to 369 U/L
y= 1.0904x - 3.7481
correlation coefficient (R^2) = 0.9943
95% confidence interval of the slope = 1.08 to 1.10;
95% confidence interval of the y-intercept = -4.8 to -2.7

Physician Office Accuracy Study at Sites 1, 2 and 3

Studies for field accuracy were performed at three external POL-type sites that were used for the precision study, plus a central laboratory to determine the correlation between the Hitachi Clinical Analyzer with the S TEST ALT and AST reagent cartridges and their respective predicate devices when using matched serum aliquots.

For the external site method comparisons studies, each POL site received 50 (ALT) to 60 (AST) blinded serum samples that were chosen to represent as full a range of ALT and AST levels as possible, and a central laboratory received a matched aliquot for every serum sample. Each sample was assayed by the Hitachi system at the POL sites, and an aliquot of each sample was assayed by a central laboratory using appropriate predicate systems (the Sekisui method for ALT and the Roche/Hitachi cobas 6000 AST assay). The results were analyzed by least squares linear regression (Hitachi results = y-axis), and the performance characteristics were as follows:

ALT (U/L)

Site #	N	Range (U/L)	Regression Equation	R^2	CI* Slope	CI* Intercept
1	49	8 to 238	y = 1.0902x - 0.7713	0.9965	1.07 to 1.11	-2.3 to 0.8
2	50	9 to 227	y = 1.0522x+ 0.1022	0.9949	1.03 to 1.07	-1.7 to 1.9
3	50	8 to 244	y = 1.1086x +0.1592	0.9933	1.08 to 1.14	-2.0 to 2.3

*95% Confidence Interval

AST (U/L)

Site #	N	Range (U/L)	Regression Equation	R^2	CI* Slope	CI* Intercept
1	64	5 to 359	y = 1.0011x –0.0894	0.998	0.99 to 1.01	-1.5 to 1.4
2	62	6 to 383	y = 1.0382x – 0.281	0.9971	1.02 to 1.05	-2.1 to 1.5
3	64	5 to 375	y = 1.0493x +0.7089	0.9973	1.04 to 1.06	-1.1 to 2.5

*95% Confidence Interval

b. Matrix comparison:

A study was performed to validate the use of sodium citrate, lithium heparin, and K3 EDTA plasma as alternatives to serum for the Hitachi Clinical Analyzer with S TEST ALT and AST reagent cartridges. Approximately 30 to 40 matched serum/plasma samples that spanned the ALT and AST dynamic ranges were assayed blinded, in singleton. The results were compared using least squares liner regression (plasma =

y-axis, serum = x-axis). Some low level samples (ALT study: Na Citrate Plasma, 5; Heparinized Plasma, 2; EDTA Plasma, 3. AST study: Na Citrate Plasma, 1; Heparinized Plasma, 1; EDTA Plasma, 0.), the results fell below their respective dynamic ranges and were excluded from the statistical analysis. This resulted in an uneven "N" in the study across the matrices. The performance characteristics were as follows.

ALT (U/L) Sample Range (serum) = 6 to 392 U/L

	Na Citrate Plasma N = 28	**Heparinized Plasma N = 31**	**K3 EDTA Plasma N= 30**
Slope (95% CIs)	0.99x (0.97 to 1.01)	1.02 (1.00 to 1.04)	1.01 (0.98 to 1.04)
y-intercept (95% CIs)	0.2 (-1.9 to 2.3)	0.2 (-1.9 to 2.3)	0.4 (-2.3 to 3.1)
r	0.998	0.998	0.997

AST (U/L) Sample Range (serum) = 5 to 369 U/L

	Na Citrate Plasma N = 38	**Heparinized Plasma N = 38**	**K3 EDTA Plasma N = 39**
Slope (95% CIs)	1.02 (1.00 to 1.04)	1.04 (1.02 to 1.06)	0.98 (0.94 to 1.02)
y-intercept (95% CIs)	-2.6 (-4.4 to -0.9)	0.6 (-1.1 to 2.2)	3.0 (-1.0 to 7.0)
r	0.999	0.999	0.992

3. Clinical studies:

   a. Clinical Sensitivity:

   Not Applicable.

   b. Clinical specificity:

   Not Applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):

   None

4. Clinical cut-off:

   Not applicable.

5. Expected values/Reference range:

   ALT - Reference range: 10-35 U/L*
   AST - Reference range: 10 to 40 U/L*

   The sponsor recommends that each laboratory determine the expected values for its particular population.

   *Tietz Fundamentals of Clinical Chemistry- 4th Edition, WB Saunders Company, Philadelphia, PA, 1996.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports substantial equivalence decision.